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Identifying key cereal aphid predators by molecular gut analysis

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Abstract

We describe polymerase chain reaction (PCR) primers for gut analysis of aphid predators. The primers amplify aphid mitochondrial COII fragments ranging in size from 77 to 386 bp. Using these primers, we were able to distinguish six species of US Great Plains cereal aphids, including two congeners, *Rhopalosiphum maidis* (Fitch) and *R. padi* (L.), and to detect them in extracts of coccinellid and chrysopid predators. We devised a protocol for deriving half-lives of detectability for the DNA of a single aphid consumed by predators maintained under simulated field dietary and temperature conditions. Using this protocol and primers that amplify a 198-bp fragment, we determined statistically different half-lives of detectability for a single *R. maidis* of 3.95 h in *Chrysoperla plorabunda* (Fitch) and 8.78 h in *Hippodamia convergens* Guerin. The detectability half-life for a 339-bp *R. maidis* fragment was statistically longer in *C. plorabunda* but not in *H. convergens*. The sensitivity of the assay for the 198-bp fragment is 10^{-7} aphid equivalents. For species-specific predator gut analysis, PCR is superior to monoclonal antibody technology, giving comparable detectability half-lives with lower expense, much shorter development times, and greater certainty of a successful outcome.

Keywords: aphid, arthropod predation, Chrysopidae, Coccinellidae, detectability half-life, PCR

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Introduction

Chemical insecticides are the predominant method of control for cereal aphids (Homoptera: Aphididae) in the US Great Plains. However, the potential of aphids to become insecticide resistant (Teetes *et al*. 1975; Rider *et al*. 1998), the poor profitability of wheat production in much of the region (Duff *et al*. 1995; Webster & Amosson 1995), and concerns about the impact of insecticides on beneficial organisms (Basedow *et al*. 1985; Matcham & Hawkes 1985) and on wildlife and the environment in general (Grue *et al*. 1988; Flickinger *et al*. 1991; Daily *et al*. 1998) all presage the eventual adoption of an integrated pest management (IPM) model for aphid management.

Although IPM may, by definition, include the use of pesticides (Kogan 1998), sustainability of cereal production systems will require reduced insecticide use and concomitant increased reliance on other IPM components. The other key components of sustainable cereal IPM programmes for the foreseeable future are resistant cultivars, modified tillage regimes and biological control using arthropod natural enemies (Burton *et al*. 1987; Reed *et al*. 1991; Rice & Wilde 1991; Farid *et al*. 1997, 1998; Brewer *et al*. 1998).

The incorporation of biological control into cereal aphid IPM programmes is hampered by a lack of basic information on the effectiveness of the natural enemy complex, including specialized parasitoids and stenophagous and polyphagous predators. The parasitoids are well known (Kring & Gilstrap 1983; Elliott *et al*. 1992, 1994; Michels & Whitaker-Deerberg 1993; Bernal *et al*. 1997; Pike *et al*. 1997, 1999), and improved methods for monitoring their impact on cereal aphid populations are under development (Zhu & Greenstone 1999; Zhu *et al*. 2000). The stenophagous predators, chiefly coccinellids and chrysopids, are also well studied (Kring *et al*. 1985; Hodek & Honek 1996; Messina *et al*. 1997; Michels *et al*. 1997;

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Elliott *et al*. 2000); some are more polyphagous than is sometimes assumed (Principi & Canard 1984; Nordlund & Morrison 1990; Triltsch 1997).

The biology of most groups of stenophagous and polyphagous predators of cereal aphids is known only in broad outline (Allen 1979; Doane & Dondale 1979; Sunderland *et al*. 1987; Nyffeler & Benz 1988; Dennis *et al*. 1990; Riedel 1991, 1995; Booij *et al*. 1995; De Snoo *et al*. 1995; Samu *et al*. 1996; Elliott *et al*. 1998; French *et al*. 1998; French & Elliott 1999; Petersen 1999). Even in the Palearctic, where cereal aphid predators have been studied most thoroughly, the importance of stenophagous and polyphagous predators in controlling cereal aphid populations is largely unknown. Nevertheless, this diverse assemblage of abundant animals may, in aggregate, impose significant mortality on aphid populations (Sunderland *et al*. 1986; Nyffeler & Benz 1988; Ekbom *et al*. 1992; Petersen 1997; Sunderland 1999). A key to their importance is their very polyphagy, which enables them to colonize fields early, persist in the absence of aphids and delay aphid population increases until specialists arrive (Chang & Kareiva 1999; Sunderland *et al*. 1999).

Even when the predator complex is well documented, it is extremely difficult to obtain data on predation rates. Arthropod predators tend to be small, cryptic and infrequent feeders, and, with very few exceptions (e.g. Coleoptera), have sucking mouthparts and hence are very difficult to study (Stuart & Greenstone 1990). Some information can be gathered by direct observation (Greenstone 1999), but gut analysis of field-collected predators is the least disruptive and most efficient means to acquire data on predation.

Gut contents have been analysed by dissection (Agarwala *et al*. 1987; Sunderland *et al*. 1987; Breene *et al*. 1990), radioactive labelling of prey (McDaniel & Sterling 1979; Breene *et al*. 1988; Godfrey *et al*. 1989), chromatography (Putnam 1965) and electrophoresis (Lister *et al*. 1987; Solomon *et al*. 1996), but the state-of-the-art for gut analysis has been serological assay. When monoclonal antibodies are used, specificity can be exquisite, extending to the species, stage and even instar level (Greenstone & Morgan 1989; Symondson & Liddell 1993; Greenstone & Trowell 1994; Hagler *et al*. 1994; Ruberson & Greenstone 1998; Agustí *et al*. 1999a; Symondson *et al*. 1999). Nevertheless, the production of monoclonal antibodies is an expensive and involved process involving scores of steps with stochastic determinants of success (Greenstone 1996), and although monoclonal antibodies were described almost 25 years ago, only a handful of entomologists have used them to study predation.

An appealing alternative is the identification of prey DNA in predator guts (Agustí *et al*. 1999b, 2000; Zaidi *et al*. 1999) There are several advantages to this approach: (i) the techniques necessary to develop molecular probes are widely known and in some cases have been subsumed into commercial kits; (ii) a variety of candidate target regions have already been sequenced in insects, providing information on their variability and hence suitability as probes; and (iii) once prey species-specific primers have been designed and published, any investigator can have them manufactured cheaply and use them in reproducible protocols. Because most cereal aphids are cosmopolitan pests, this makes the research results useful to entomologists worldwide.

We targeted the COII gene in our research. As a mitochondrial gene, it occurs as multiple copies per cell, which increases the likelihood of successful amplification in gut extracts. It also offers various levels of variability (Zhang & Hewitt 1996), allowing closely related species to be separated. Finally, sequences are already available for several aphid species (Rouhbakhsh *et al*. 1996; Sunnucks & Hales 1996).

Our objectives in this research were to: (i) develop species-specific polymerase chain reaction (PCR) primers for the principle cereal aphid pests of the US Great Plains; (ii) show that we could use them to detect aphid DNA in the guts of representative cereal aphid predators; and (iii) develop a realistic and manageable protocol for determining aphid DNA detectability half-lives in the predators.

Materials and methods

Insects

Aphids, from colonies at the USDA-ARS Plant Science Research Laboratory in Stillwater, Oklahoma, were maintained at ≈25 °C and a photoperiod of 12 : 12 (light : dark) on barley, wheat or sorghum as appropriate. We developed specific COII primers for six members of the US Great Plains cereal aphid (Homoptera: Aphididae) complex: greenbug (Biotype E), *Schizaphis graminum* (Rondani); Russian wheat aphid, *Diuraphis noxia* (Mordvilko); bird cherry-oat aphid, *Rhopalosiphum padi* (L.); corn leaf aphid, *R. maidis* (Fitch); yellow sugarcane aphid, *Sipha flava* (Forbes); and English grain aphid, *Sitobion avenae* (F.).

Convergent lady beetles, *Hippodamia convergens* Guerin, and sevenspotted lady beetles, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) were collected in Payne Co., Oklahoma. Mated pairs were maintained at 24 °C and a photoperiod of 16 : 8 (light : dark) on pea aphids, *Acyrthosiphon pisum* (Harris) and honey-wheat-yeast supplement; additional *H. convergens* were supplied by Gardens Alive! (Lawrenceburg, IN, USA). Common green lacewings, *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae), were purchased as eggs from Rincon-Vitova Insectaries (Ventura, CA, USA); larvae were maintained at 20 °C and a photoperiod of 11 : 13 (light : dark) on *D. noxia* or *S. graminum*, and eggs of Agonoumis grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae).

Table 1 Aphid and predator primer sequences (5′–3′)

Primer	Sequence
COIIF	CATTCATATTCAGAATTACC
COII318F	AGAAAT/CTCA/CCCATTAATAGAACA
COIIR	GAGACCATTACTTGCTTTCAGTCATCT
COIIFC440	CACCGATTTTTATTAGAAGGTCA
COII860R	CCACAAATTTCTGACCATTG

DNA extraction

We modified the methods of Zhu & Greenstone (1999) to extract total insect DNA. Insects were placed individually in 1.5-mL microcentrifuge tubes and homogenized using a battery-powered homogeniser (Midwest Scientific, St. Louis, MO, USA) in 100 μL or 500 μL, for aphids and predators, respectively, of isolation buffer containing 0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl (pH 9.1), 0.05 M EDTA, 1% SDS and 20 μg/mL RNAase A. The homogenate was vortexed briefly and incubated for 30 min at 65 °C. The solution was transferred to a new tube and extracted once with 1 vol. of chloroform/isoamyl alcohol (24 : 1). One-tenth volume of 3.0 M sodium acetate and 2 vol. of ice-cold 100% EtOH were added to the tube. DNA was then pelleted by centrifugation, dried and resuspended in 200 μL distilled water.

PCR amplification, purification and sequencing of COII

Aphid DNA fragments were amplified using forward primers COIIF (Stern 1994) and COII318F (Table 1), and reverse primer COIIR (Rouhbakhsh *et al*. 1996); coccinellid and chrysopid fragments were amplified using forward primer COIIFC440 and reverse primer COII860R (Table 1). COIIFC440 and COII860R were designed by aligning published *Adalia bipunctata* (L.) and *Chrysomela tremula* DNA sequences from GenBank (Accession nos M83965 and AF014642, respectively); COII318F was designed by aligning all of the known aphid DNA sequences. PCR products were separated on a 1.0% low melting point agarose gel. DNA fragments were sliced from the gel and extracted using a Wizard PCR Preps DNA Purification System (Promega, Madison, WI, USA). Purified DNA fragments were sequenced directly using an automated sequencer located at the Recombinant DNA/Protein Resource Facility, Oklahoma State University, Stillwater. Reamplified products were cloned into a TA cloning vector (pCR®2.1-TOPO™, Invitrogen Corporation, Carlsbad, CA, USA) and sequenced on a Perkin-Elmer Applied Biosystems 373A automated DNA sequencing system using the Prism™ Ready Reaction Dyedeoxy™ Terminator Cycle Sequencing kit (Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA).

GenBank Accession nos for the COII sequences are AF254089–AF254094 (aphids) and AF254095–AF254097 (predators).

Primer design and PCR amplification of aphid and predator DNA

After obtaining all of the aphid and predator COII DNA sequences, we used GCG Wisconsin Package UNIX version 10 (Genetics Computer Group, Madison, WI, USA) for alignment and analysis. Primers were designed to separate all aphid species from one another and from each predator species. We used the single base-detection technique (Kwok *et al*. 1990) to design primers for separating aphid species. PCR reactions (25 μL) contained 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 1.0 μM of each primer, 50 mM KCl, 0.1 mM of each dNTP, 0.05 U/μL of *Taq* DNA polymerase (Promega) and 2 μL of template containing 10–100 ng DNA, and were performed in a PTC-100 thermocycler (MJ Research, Watertown, MA, USA); on the basis of trial-and-error optimization studies, *C. plorabunda* DNA was diluted 10× further than the other species in distilled water prior to PCR. DNA was denatured for 3 min at 94 °C, followed by 35 amplification cycles, with 30 s denaturing at 94 °C, 30 s annealing at 55–57 °C, depending on the primers (Innis *et al*. 1990), and 1 min extension at 72 °C. DNA was finally extended for 2 min at 72 °C after amplification. PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and photographed under UV light. Because larger fragments may be digested more quickly than smaller ones (Agustí *et al*. 1999b; Zaidi *et al*. 1999), we designed primer pairs to amplify fragments between 77 and 386 bp.

Feeding studies

In order to determine whether we could detect the DNA of specific aphid species in the guts of predators, *H. convergens* and *C. septempunctata* third instars and adults were removed from the colonies, housed individually in 2-dram shell vials with moistened cotton plugs, and fed *R. padi* or *R. maidis ad libitum*. After 2 days, those that had consumed at least 10 aphids were frozen. Two to five third instars or adults were individually assayed with *R. padi* and *R. maidis* primers.

In order to determine the half-life (the time after which only half of the meals eaten can be detected; Greenstone & Hunt 1993) of detectability of the DNA of a single aphid meal, we set up groups of 120–150 *H. convergens* and *C. plorabunda* as eggs. Resulting third instars were housed individually in 2-dram vials with moistened

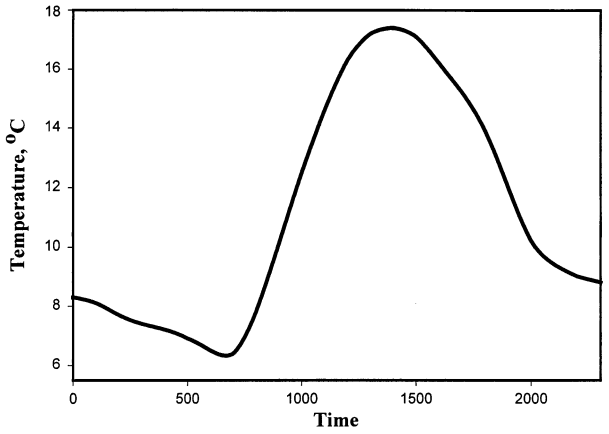

Fig. 1 Mean hourly temperature profile in the canopy of a wheat field in Chickasha, Oklahoma, from 15 March to 14 April 1999.

cotton plugs and placed in a Conviron (Pembina, ND, USA) I23 incubator programmed to reproduce the mean hourly temperature profile measured 10 cm above ground in the canopy of a wheat field in Chickasha, Oklahoma, from 15 March to 14 April 1999 (Fig. 1); this height is well within the vertical distribution of *H. convergens* (Ewert & Chiang 1966). The interval over which temperatures were averaged contained peaks in coccinellid and chrysopid densities in the same field (B. Wade French, USDA-ARS Brookings, SD, personal communication).

All food was removed 24 h before the experiment. At 10.00 hours on the following day, the larvae were each fed a single *R. maidis* and observed until they had consumed it. Those that had not consumed the aphid within 1 h were dropped from the experiment. Twenty animals of each predator species, designated as the 0 h group, were immediately frozen at –20 °C; those remaining were offered five *R. padi* and returned to the incubator. At 4, 8, 12 or 16 h postfeeding, 20 per group were removed from the incubator and frozen. Thus, each animal had an opportunity to feed on a total of six aphids (one *R. maidis* and up to five *R. padi*). This represents 15 and 30% of a daily *ad libitum* diet for *C. plorabunda* and *H. convergens*, respectively (Burke & Martin 1956; Michels & Biehle 1991). Because predation is rarely observed in the field

Table 2 Aphid species-specific primer sequences (5′–3′), optimal PCR annealing temperatures, and amplified fragment sizes

Name	Primer sequence	Annealing temp. (°C)	Target species	Size (bp)
BcoaCOIIF1	TATCTGAACTACAACTCCAC	55	*Rhopalosiphum padi*	331
BcoaCOIIR1	GGATTGCATCAATTTTAATAGCTAAA			
BcoaCOIIF4	TCATTCATGAACAATTCCAAG	55	*R. padi*	148
BcoaCOIIR2	GAATAGGTATAAATCTGTGATTAATA			
BcoaCOIIF3	TTCGACTCTTAATTTCATCA	55	*R. padi*	77
BcoaCOIIR1	GGATTGCATCAATTTTAATAGCTAAA			
ClaCOIIF	CCAATTCTAACAATTAAAATTTTTGGA	57	*R. maidis*	198
ClaCOIIR1	GAATAACATCATCTGATGAAATTAAA			
ClaCOIIF	CCAATTCTAACAATTAAAATTTTTGGA	57	*R. maidis*	246
ClaCOIIR2	CTGGGATTGCATCAATTTTAATA			
ClaCOIIF	CCAATTCTAACAATTAAAATTTTTGGA	57	*R. maidis*	339
ClaCOIIR3	GTATAAATCTATGATTAATTCCACAA			
GbCOIIF2	GATGTTATTCACTCATGAACA	55	*Schizaphis graminum*	166
GbCOIIR2	GATTCAATTTGAATAGGTATAAAA			
GbCOIIF1	TTTGAACTACAACTCCTCCA	55	*S. graminum*	386
GbCOIIR1	GTCCAAAATATATTCCTGGG			
GbCOIIF2	GATGTTATTCACTCATGAACA	55	*S. graminum*	111
GbCOIIR1	GTCCAAAATATATTCCTGGG			
RwaCOIIF1	TACCATCTTTACACCTATTA	57	*Diuraphis noxia*	348
RwaCOIIR1	CATTGTCCAAAATATAATCCA			
RwaCOIIF2	CCGATTATTAATTTCATCAGA	57	*D. noxia*	137
RwaCOIIR1	CATTGTCCAAAATATAATCCA			
RwaCOIIF3	TGAACTATCCCAAGATTAGC	57	*D. noxia*	100
RwaCOIIR1	CATTGTCCAAAATATAATCCA			
EgaCOIIF1	TATTTGAACTACAACTCCTC	55	*Sitobion avenae*	231
EgaCOIIR	AGTTTTATTGTCTACTTCAATTAAA			
EgaCOIIF2	AGATGAAATTAAATGTCCCA	55	*S. avenae*	159
EgaCOIIR	AGTTTTATTGTCTACTTCAATTAAA			
YscaCOIIF1	TATTTGAACAGCAATTCCTC	55	*Sipha flava*	326
YscaCOIIR	GCATCAATTTTAATTCCTAATCTG			
YscaCOIIF2	TTATTGCTTTACCTTCACTA	55	*S. flava*	291
YscaCOIIR	GCATCAATTTTAATTCCTAATCTG			
Aphid F	TTTCCGATTAATTGAAGTAG	52	All aphid spp.	181
Aphid R	ATTCCTGGTCGGTTTATAAA			

(Elliott *et al*. 2000), and coccinellids, at least, are not feeding maximally in the field (Obrycki *et al*. 1998), this is a reasonable simulation of aphid consumption under field conditions.

Total DNA was extracted from each predator and subjected to PCR as described above, using *R. maidis* primers ClaCOIIF and ClaCOIIR1 (Table 2). We determined half-lives of aphid DNA detectability by subjecting data on the proportion of predators positive for *R. maidis* DNA to Probit analysis using PROC PROBIT in PC SAS Version 6.11 (SAS Institute 1996). Half-lives for *R. maidis* in *C. plorabunda* and *H. convergens* were compared using the methodology of Robertson & Priesler (1992).

Sensitivity

We determined the sensitivity for aphid DNA by subjecting serial 10-fold dilutions of *R. maidis* DNA, in the standard dilution of extracted DNA of each predator (10^{-2} larval equivalents of *H. convergens* and 10^{-3} larval equivalents of *C. plorabunda*), to PCR as described above.

Results

Sequences, optimal annealing temperatures, and fragment sizes for all primer pairs are shown in Table 2. Using these primers, we were able to detect all aphids species specifically (Figs 2 and 3). We could also distinguish

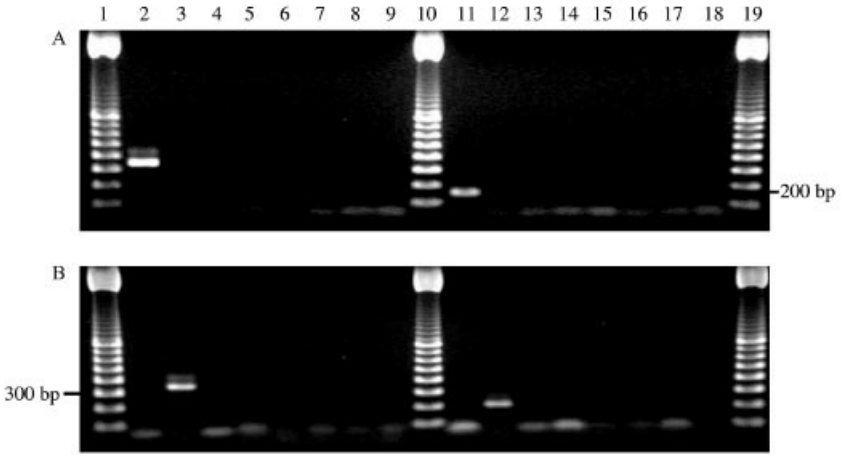

Fig. 2 PCR amplification of *Rhopalosiphum padi* and *R. maidis*. (A) *R. padi* primers used: BcoaCOIIF1 and BcoaCOIIR1 (lanes 2–9), BcoaCOIIF4 and BcoaCOIIR2 (lanes 11–18). (B) *R. maidis* primers used: ClaCOIIF and ClaCOIIR3 (lanes 2–9), ClaCOIIF and ClaCOIIR1 (lanes 11–18). Lanes 1, 10 and 19: 100 bp DNA ladder; lanes 2, 11: *R. padi*; lanes 3, 12: *R. maidis*; lanes 4, 13: *Schizaphis graminum*; lanes 5, 14: *Diuraphis noxia*; lanes 6, 15: *Sitobion avenae*; lanes 7, 16: *Sipha flava*; lanes 8, 17: *Coccinella septempunctata*; lanes 9, 18: *Hippodamia convergens*.

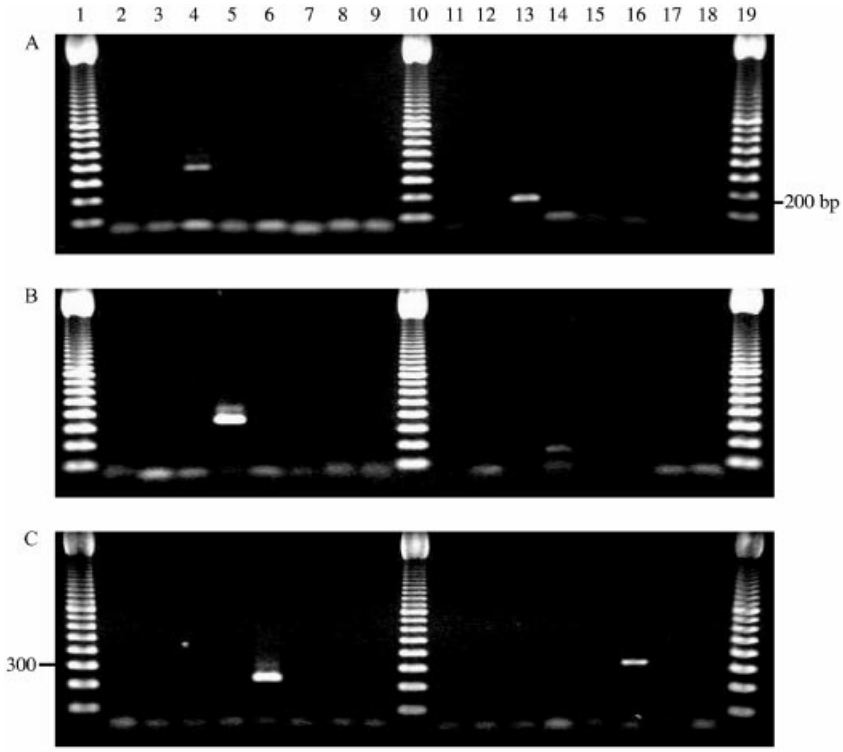

Fig. 3 PCR amplification of non-*Rhopalosiphum* aphid species. (A) *Schizaphis graminum* primers used: GbCOIIF1 and GbCOIIR1 (lanes 2–9), GbCOIIF2 and GbCOIIR2 (lanes 11–18). (B) *Diuraphis noxia* primers used: RwaCOIIF1 and RwaCOIIR1 (lanes 2–9), RwaCOIIF2 and RwaCOIIR1 (lanes 11–18). (C) *Sitobion avenae* primers used: EgaCOIIF1 and EgaCOIIR (lanes 2–9); *Sipha flava* primers used: YsaCOIIF1 and YsaCOIIR (lanes 11–18). Lanes 1, 10 and 19: 100 bp DNA ladder; lanes 2, 11: *R. padi*; lanes 3, 12: *R. maidis*; lanes 4, 13: *S. graminum*; lanes 5, 14: *D. noxia*; lanes 6, 15: *S. avenae*; lanes 7, 16: *S. flava*; lanes 8, 17: *Coccinella septempunctata*; lanes 9, 18: *Hippodamia convergens*.

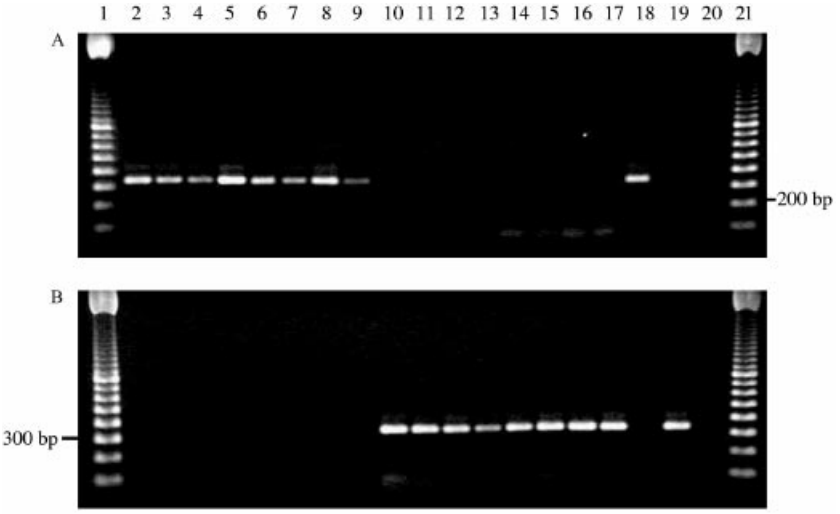

Fig. 4 PCR amplification of *Hippodamia convergens* fed *Rhopalosiphum padi* and *R. maidis*. (A) *R. padi* primers used: BcoaCOIIF1 and BcoaCOIIR1. (B) *R. maidis* primers used: ClaCOIIF and ClaCOIIR3. Lanes 1, 21: 100 bp DNA ladder; lanes 2–5: adults fed *R. padi*; lanes 6–9: larvae fed *R. padi*; lanes 10–13: adults fed *R. maidis*; lanes 14–17: larvae fed *R. maidis*; lane 18: *R. padi*; lane 19: *R. maidis*; lane 20: *H. convergens*.

the two *Rhopalosiphum* spp. from each other after consumption by *Hioppodamia convergens* (Fig. 4) and *Coccinella septempunctata* (data not shown). Furthermore, we could detect DNA of any of the six aphid species in the gut of *H. convergens* and *Chrysoperla plorabunda* using an all-aphid primer pair (Fig. 5).

The remains of a single *Rhopalosiphum maidis* could be detected for several hours after feeding by both *H. convergens* and *C. plorabunda* (Figs 6 and 7); because many of these predators consumed *R. padi* immediately afterwards, the assay can specifically detect target DNA in the presence of competing congeneric DNA. The proportions of animals positive for *R. maidis* DNA data in the half-life experiment were well described by the Probit model, with likelihood ratio goodness-of-fit test statistics of 2.8502 ($P = 0.4153$) for *C. plorabunda* and 4.4554 ($P = 0.2163$) for *H. convergens*. The slopes for both predator species were significantly different from zero ($P < 0.0001$). The half-life of detectability for the DNA of a single corn leaf aphid, using primers for the 198-bp amplified fragment, was 3.95 h for *C. plorabunda* and 8.78 h for *H. convergens* (Fig. 7). These half-lives are significantly different with $P < 0.0001$ (Robertson & Priesler 1992).

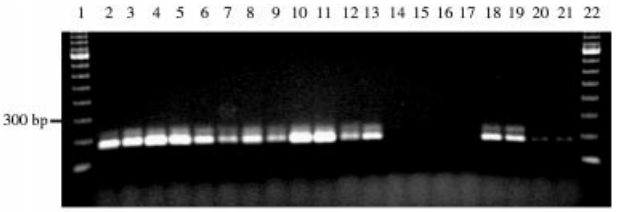

Fig. 5 PCR amplification of all aphid species using primers Aphid F and Aphid R. Lanes 1 and 22: 100 bp DNA ladder, lanes 2, 3: *Diuraphis noxia*; lanes 4, 5: *Schizaphis. graminum*; lanes 6, 7: *Sitobion avenae*; lanes 8, 9: *Sipha flava*; lanes 10, 11: *Rhopalosiphum maidis*; lanes 12, 13: *R. padi*; lanes 14, 15: *Hippodamia convergens*; lanes 16, 17: *Coccinella plorabunda*; lanes 18, 19: *H. convergens* fed with *R. maidis*; lanes 20, 21: *C. plorabunda* fed with *R. maidis*.

Fragment size did not affect detectability half-life in *H. convergens*, but the largest fragment (339 bp) had a statistically shorter half-life than the two smaller fragments in *C. plorabunda* (Table 3). The sensitivity of PCR for the 198 bp fragment of *R. maidis* DNA in DNA extracts of both predator species is 10^{-7} aphid equivalents (Fig. 8).

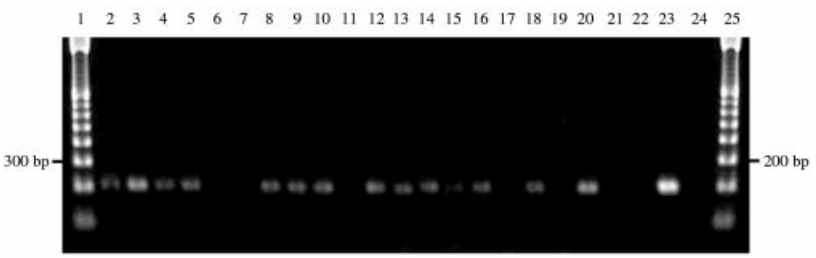

Fig. 6 PCR amplification, using *Rhopalosiphum maidis* primers ClaCOIIF and ClaCOIIR1, of *Hippodamia convergens* third instars fed one *R. maidis*, 8 h after ingestion. Lanes 1 and 25: 100 bp DNA ladder; lanes 2–21: *H. convergens* fed one *R. maidis*; lane 22: *R. padi*; lane 23: *R. maidis*; lane 24: *H. convergens*.

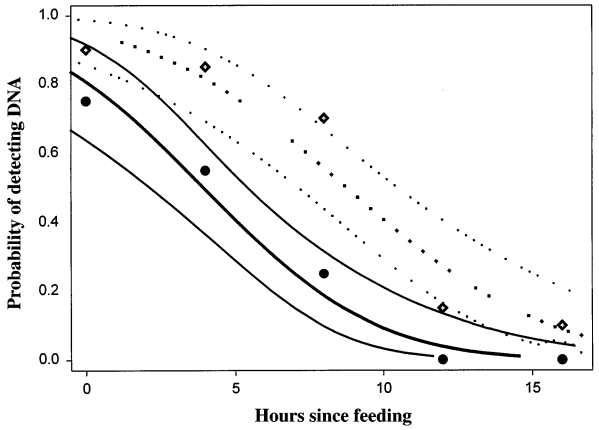

Fig. 7 The probability of detecting DNA from a single *Rhopalosiphum maidis*, vs. hours since feeding. Lines are fitted Probit model with 95% fiducial limits. Circles and solid lines, *Chrysoperla plorabunda*; diamonds and dotted lines, *Hippodamia convergens*. $N = 20$ individual predators assayed for each point.

Table 3 Half-lives of detectability for the DNA of a single *Rhopalosiphum maidis* as a function of DNA fragment size

Predator species	Amplified fragment size (bp)	Half-life (h)	Slope (SE)
Chrysoperla plurabunda	339	2.56[a]	0.4294 (0.0934)
	246	3.81[b]	0.2298 (0.0435)
	198	3.95[b]	0.2193 (0.0412)
Hippodamia convergens	339	7.72[a]	0.1724 (0.0292)
	246	8.22[a]	0.1895 (0.0314)
	198	8.78[a]	0.1877 (0.0308)

Within a predator species, half-lives followed by the same letter are not significantly different ($P > 0.05$).

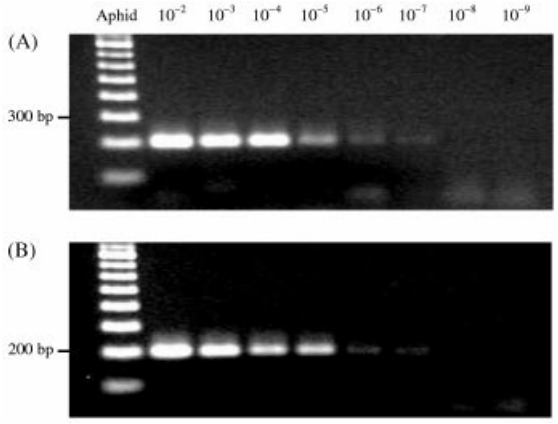

Fig. 8 Sensitivity of PCR, determined by titration of *Rhopalosiphum maidis* DNA in DNA of *Hippodamia convergens* (10^{-2} larval equivalents) (A) and *Chrysoperla plorabunda* (10^{-3} larval equivalents) (B).

Discussion

Our results show that we are able to identify the DNA of specific cereal aphid species in the guts of arthropod predators for ecologically relevant intervals following ingestion. By focusing on two aphid congeners, we have made a very stringent case for specificity. We detected aphid DNA in the guts of predators from two different insect orders, and devised a workable protocol for determining significant aphid DNA detectability differences under simulated field temperature and feeding conditions. Our all-aphid primer pair will enable us to save time, materials and expense, by subjecting field-collected predators to a two-step assay protocol: (i) subject all predator extracts to all-aphid PCR; (ii) subject only extracts positive in the first assay to species-specific aphid PCR.

Detectability half-lives for a single prey item of the order we report here are comparable with those achieved by the gold standard for gut analysis, monoclonal antibody detection of prey antigens (Greenstone & Hunt 1993; Symondson *et al*. 1997; Agustí *et al*. 1999a). Detectability half-lives are necessary because mere determination of the proportion of predator individuals positive for prey DNA is not a reliable indicator of the relative importance of any given predator taxon. For example, *Chrysoperla plorabunda* has a half-life (3.95 h) for detectability of *Rhopalosiphum maidis* DNA that is only 0.45 that (8.78 h) of *Hippodamia convergens*. Consequently, the consumption of a single *R. maidis* is 2.2 times as likely to be detected in an *H. convergens* individual as in a *C. plorabunda* individual, and the raw proportions must be corrected to reflect this difference. We may expect to find dramatic differences in detectability half-lives as more predator taxa are studied. For example, in the analogous case of detecting protein antigens in serological predator gut analysis, staphylinid beetles appear to have short detectability half-lives (Sunderland *et al*. 1987), and spiders much longer ones (Ragsdale *et al*. 1981; Greenstone 1983).

Because DNA detectability decays exponentially, there is no finite detectability period. However after four and five half-lives, roughly 94 and 97%, respectively, of all meals will have been detected. This may prove useful in designing sampling plans in relation to predator diel feeding cycles.

In practice, the predator species with the shortest half-life for a particular prey DNA would be given a detectability weighting of 1.0, and the half-lives of all other predator species would be divided by the benchmark half–life to give a detectability weighting for each predator species. In this two-species example, *C. plorabunda* would be assigned a weight of 1.0 for detectability of *R. maidis* DNA, whereas *H. convergens* would receive a weight of 0.45. Densities for each predator species, determined by absolute methods (e.g.

Sunderland & Topping 1995; Michels *et al*. 1997), would be multiplied by these weightings to determine an importance value for each predator species consuming the pest.

These importance values will identify species that are apt to consume large numbers of the pest. They could then be used to focus research to enhance the numbers of these key species, for example by microhabitat manipulation (Riechert & Bishop 1990; Rice & Wilde 1991; Brust 1994; Samu *et al*. 1999), as part of an IPM strategy.

Other uses of predation data include the construction of functional response curves (Provencher & Coderre 1987; O'Neill 1997) and pest life tables (Yamanaka *et al*. 1972; Hogg & Nordheim 1983). Such uses require an estimate of the number of prey items per gut represented by a positive assay. Derivation of such estimates from qualitative gut assay data is not straightforward (Greenstone 1996). Although a variety of approaches have been proposed (Nakamura & Nakamura 1977; Greenstone 1979; Lister *et al*. 1987; Sopp *et al*. 1992), they have not been verified experimentally. Furthermore, one must be aware of the routes other than predation by which insect remains can come to reside in the gut of another arthropod (Sunderland 1996).

Agustí *et al*. (1999b, 2000) and Zaidi *et al*. (1999) found that larger fragments became undetectable in the gut more rapidly than smaller ones. Working with a smaller range of fragment sizes than those authors, we found no differences in detectability half-lives for fragments of 246 bp and shorter.

Numerous DNA regions have been sequenced in many insect taxa. For predator gut analysis, one looks for DNA sequences represented by as many copies as possible so that some target survives as long as possible under the onslaught of digestion. Therefore, multiple copy sequences are the best candidates. Zaidi *et al*. (1999) used esterase genes believed to be present in ≈40–50 copies per cell. We chose mitochondrial genes because they are typically present as hundreds to thousands of copies per cell (Hoy 1994).

With this report and those of Agustí *et al*. (1999b, 2000) and Zaidi *et al*. (1999), predator gut analysis by PCR should be considered an established technology. The only rival PCR has for sensitivity and specificity in gut analysis is monoclonal antibody technology. PCR has the advantage of requiring much less time and expense in development, plus reasonable assurance that if a well-characterized DNA region is selected, a useful result will be achieved in a few months vs. many months to years for monoclonal antibodies (Greenstone 1996; Zaidi *et al*. 1999).

The equipment required for developing PCR primers consists of a thermocycler, power supply and gel apparatus for electrophoresis of PCR products, available for <$5000. One must also sequence DNA regions and manufacture the oligonucleotide primers, but these tasks are performed economically by centralized institutional facilities ($10–20 per item). Labour and supplies costs are significant but relatively predictable and limited due to the rapidity with which useful sequences can be discovered and specific primers made for them.

The development of monoclonal antibodies entails up-front equipment expenditures on the order of $20 000 for a CO_2 incubator, laminar-flow hood, liquid nitrogen freezer and ELISA plate reader. The supplies and labour costs vary, depending on how long it takes to find a useful hybridoma line. Monoclonal antibody development entails considerable labour, including some outside of normal working hours because hybridoma lines mature at different rates and have very narrow time windows for evaluation. The entire process (Greenstone 1996) will take from 6 months if one is extremely lucky and finds it on the first attempt, to several years if one is not. For example, the first monoclonal antibody to be used in arthropod predator gut analysis was obtained in only two fusions (Greenstone & Morgan 1989), but the same laboratory, despite several more years experience, required seven fusions to develop a species-specific monoclonal antibody against another stage of the same prey species (Greenstone & Trowell 1994; Greenstone 1995).

Once the specific monoclonal antibody or primers are obtained, predators must be extracted and assayed. To our knowledge, a cost comparison between monoclonal antibody-based immunoassay and PCR has not been attempted. Here we assume that individual predator assays are not replicated and that all supplies are purchased in bulk. We assume that buffer, predator extraction and labour costs are the same.

ELISA is performed in 96-well plates. Because of high variability among plates, and unreliable readings on the plate perimeter, each plate must contain its own standards, and only the 60 inner wells of each plate can be used (Kricka *et al*. 1980; Fenlon & Sopp 1991). Each sample well's absorbance must be read quantitatively, then compared statistically with the mean of a series of negative controls. If 10 controls minimize the probability of a Type I error to an acceptable level (Schoof *et al*. 1986), 50 wells remain for individual predator samples. A monoclonal antibody may be harvested from hybridoma tissue culture supernatant at a cost of $650/g (M. H. Greenstone, unpublished). If one uses 100 μg of antibody per individual assay, and sets up 10 plates simultaneously to minimize the number of pipette tips, then the cost to run an individual predator extract using ELISA is ≈$0.21.

More work is required before high throughput PCR assays can be run. It is, however, already possible to run PCR in microplates rather than individual tubes. In a 96-well plate all wells can be used because there are no edge effects. If reactions are run in a 10-μL volume and, for example, six wells are reserved for various controls, then

an individual predator can be assayed for ≈$0.28, which is competitive with ELISA.

One disadvantage of PCR in comparison with monoclonal antibody technology is the inability to achieve stage or instar level specificity (cf. Greenstone & Morgan 1989; Hagler *et al*. 1994; Greenstone 1995) because of the presence of DNA in all tissues of all life stages. Such specificity might be achieved by reverse-transcriptase PCR, enabling the detection of mRNAs expressed at different developmental periods in the life of the insect. Given the ubiquity of RNAases in animal tissues, however (Sambrook *et al*. 1989), mRNAs could have shorter half-lives in the gut than DNAs. Whether this is true would have to be determined empirically in each case.

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